

Genetic Analyses of HIV-1 Strains Transmitted from Mother to Child in Northern Vietnam

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Abstract

We previously reported mother-to-child transmission of HIV-1 in nine (6.7%) of 135 children on nevirapine prophylaxis in Vietnam. In the current study, we investigated the appearance and profile of antiretroviral drug (ARV) resistance mutations, the predicted coreceptor usage, and the genetic diversity of HIV-1 strains isolated from the eight pairs of HIV-1-infected mothers and their children, who were followed up to 12 months after birth. Portions of the *pol* and *env* C2V3 regions of the HIV-1 strains were analyzed genetically. HIV-1 CRF01_AE RNA was detected in four (50%) children at delivery. Y181C, a nevirapine resistance mutation, appeared in two (25%) children 1 and 3 months after birth, respectively. No ARV resistance mutation was detected in the mothers, though three mothers were on ARV prophylaxis. Five mothers and their children harbored CCR5-tropic (R5) viruses. Two mothers harbored both R5 and CXCR4-tropic (X4) viruses, but their children harbored only R5 viruses even though the X4 viruses were dominant in the mothers. In the remaining one mother, HIV-1 RNA was not amplified and her child harbored both R5 and X4 viruses at birth, but only X4 virus 12 months after delivery. The infants' viruses were more homogeneous than their mothers' viruses (mean distance: 0.5% vs. 1.1%, respectively). This is the first molecular epidemiological study of vertical HIV-1 infections in Vietnam. These findings may provide useful knowledge for the prevention of mother-to-child transmission of HIV-1 and the antiretroviral treatment of children in Vietnam.

Introduction

VERTICAL TRANSMISSION OF HUMAN immunodeficiency virus type 1 (HIV-1) accounts for the majority of pediatric AIDS cases in the world. In 2012, more than 260,000 children less than 15 years of age were newly infected with HIV-1, more than 95% of these cases via mother-to-child transmission (MTCT).^{1,2} MTCT of HIV-1 can occur *in utero*, intrapartum, and postpartum through breastfeeding. Perinatally acquired infections account for the majority of all HIV-1 cases in children, with an estimated MTCT rate of 14–48% without prevention.^{3–6} However, the timing and actual mechanisms of perinatal transmission are not clear.

The chemokine receptors CCR5 and CXCR4 are the main coreceptors for HIV-1 entry into target cells.⁷ Based on chemokine coreceptor usage, HIV-1 can be classified as a CCR5-tropic (R5), CXCR4-tropic (X4), or dual tropic (R5X4) virus.⁸ A strong selection for viral variants using CCR5 as a coreceptor occurs during transmission, as most children are infected with R5 viruses.^{9–12}

Prevention of mother-to-child transmission (PMTCT) of HIV-1 is one of the key components of the worldwide response to the pandemic and is based on the prophylactic use of antiretroviral drugs (ARVs). In developed countries, MTCT of HIV-1 has been reduced to less than 2% by combination antiretroviral therapy (ART), selective caesarian delivery, and avoidance of breastfeeding.^{13,14} In developing countries, single-dose nevirapine given to mothers infected with HIV-1 and their children in the intrapartum and immediate postnatal period is the most common intervention to reduce the MTCT of HIV-1. Single-dose nevirapine can reduce the risk of MTCT of HIV-1,¹⁵ but it may lead to the emergence of nevirapine-resistant HIV-1 variants in children who are infected with HIV-1 despite the prophylaxis.¹⁶ One study of HIV-1-infected Ugandan women and their children who received single-dose nevirapine to prevent MTCT showed nevirapine resistance mutations in 11 of 24 (46%) children by 6–8 weeks of age.¹⁶

In Vietnam, the first pregnant woman infected with HIV-1 was reported in 1993. The number of HIV-1-infected pregnant women increased from 4,000 in 2007 to almost 5,000 in

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2012, and the number of children living with HIV-1 increased from 3,750 in 2007 to 5,700 in 2012.^{17–19} PMTCT was officially introduced in Vietnam in 2006. According to the guidelines of the Vietnamese government in 2005, all HIV-1-infected pregnant women and their children should receive single-dose nevirapine free of charge.²⁰ However, of the HIV-1-infected pregnant women in Vietnam, only 9.2% in 2006, 32.3% in 2009, and 44.0% in 2011 had access to the PMTCT program, and 27.9% received single-dose nevirapine in 2011.²¹ Most of the women who had access to the PMTCT program resided in urban areas, such as Hanoi and Haiphong.^{18,21} One study reported that 7.6% of the HIV-1-infected pregnant women were on combination ARVs, 51.9% on single-dose nevirapine, and the remaining 40.4% without ARVs; only 46.2% of their children received single-dose nevirapine for PMTCT in 2008.¹⁸

MTCT of HIV-1 was observed in nine (6.7%) of the 135 children, who were all on single-dose nevirapine prophylaxis and were born to mothers with and without ARV prophylaxis between 2004 and 2006 in Northern Vietnam.²² However, limited data are available on the factors associated with vertical transmission of HIV-1, such as the transmission of drug-resistant HIV-1 and selection based on coreceptor usage in Vietnam.

In the present study, we investigated the appearance and profile of drug resistance-associated mutations in infants under nevirapine prophylaxis, the predicted coreceptor usage, and the genetic diversity of HIV-1 strains in mothers and their infants in Hanoi and Haiphong, Northern Vietnam.

Materials and Methods

Study subjects

A total of 135 HIV-1-infected mothers and their children admitted to the Obstetrics and Gynecology hospitals in Hanoi and Haiphong, Northern Vietnam, were enrolled in our previous study.²² Blood samples were collected from the mothers on the day of delivery and from their infants within 1–2 days after birth between 2004 and 2006. Blood samples were also collected from the infants 1, 3, 6, and/or 12 months after birth, although some mother-and-child pairs, especially those who lived far from the hospital, could not come to the hospital at all the sample collection times. Plasma was separated from the blood samples within 8 h of collection and stored at -80°C until analysis.

For the current study 38 mother-and-child pairs including one with a child vertically infected with HIV-1 were excluded for the lack of their mothers' plasma samples. Plasma samples from the remaining 97 pairs were still available and transferred to Kanazawa University for further analyses (Fig. 1). Of the 97 children samples eight were confirmed to be positive for HIV-1 RNA (Table 1). Of the eight HIV-1-infected children, one (HP25) was born to a mother given zidovudine/lamivudine/nelfinavir 1–3 weeks before delivery, two (HN51 and HN88) were born to mothers given single-dose nevirapine at delivery, and five (HN23, HN46, HN47, HN60, and HP13) were born to mothers without any prophylaxis. After birth, all children were given single-dose nevirapine (2 mg of liquid nevirapine/kg body weight) within 48 h of delivery and fed formula, and one child (HP25) whose mother received combination ART before delivery was given liquid zidovudine for 7 days after birth according to the Vietnamese national guidelines.²⁰ All of the plasma samples

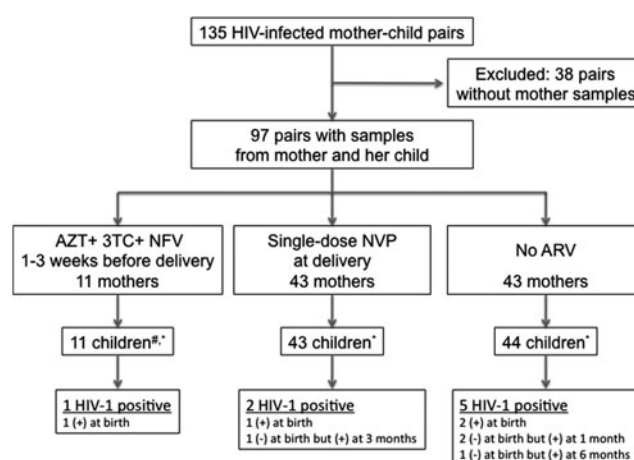


FIG. 1. Mother-to-child transmission in the study group. AZT, zidovudine; 3TC, lamivudine; NFV, nelfinavir; NVP, nevirapine. *AZT liquid was administered for 7 days after birth. All children were given single-dose liquid nevirapine within 48 h of birth and fed with formula.

collected from the eight HIV-1-infected children within 1–2 days after birth were available, but only a part of the samples collected from these children 1, 3, 6, and/or 12 months after birth were available as shown in Table 1. None of the children visited any other institutes or started ARV during the follow-up.

This study was approved by the ethical committees of the Hanoi National Hospital of Pediatrics, the National Institute of Hygiene and Epidemiology, the Obstetrics and Gynecology hospitals in Vietnam, and Kanazawa University in Japan.

RNA extraction, RT-PCR, cloning, and sequencing

RNA was extracted from 100 μl of plasma using the SMITEST EX-R&D nucleotide extraction kit (Genome Science Laboratories, Fukushima, Japan) according to the manufacturer's instructions. One-step reverse transcriptase polymerase chain reaction (RT-PCR) (SuperScript III One-step RT-PCR system with Platinum Taq DNA polymerase, Invitrogen, Carlsbad, CA) and nested PCR with AmpliTaq Gold (Applied Biosystems, Japan) or KOD FX (Toyobo, Osaka, Japan) were used to amplify HIV-1 *pol* and *env*.

The HIV-1 *pol*-PR region (coding amino acids 1 to 99) was amplified by nested RT-PCR with the primers DRPRO5/DRPRO2L in the first round and DRPRO1M/DRPRO6 in the second round. The HIV-1 *pol*-RT region (coding amino acids 1 to 220) was amplified using the primers DRRT1L/RTout in the first round and DRRT7L/DRRT6L in the second round. To amplify HIV-1 *pol*-RT, the primer pairs RT18/K104 and K101/K102 were used in the first and second round, respectively, when the PCR reaction with the previous primer pairs failed.^{23–25} RT-PCR was performed with one cycle at 55°C for 30 min and one cycle at 94°C for 2 min, then 40 cycles at 94°C for 15 s, 55°C (for DRPRO5/DRPRO2L and DRRT1L/RTout primer pairs) or 50°C (for the other primer sets) for 30 s, and 68°C for 1 min, with a final extension of 68°C for 5 min.^{23–25} Nested PCR for *pol*-PR was performed with one cycle at 95°C for 10 min, followed by 40 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 10 min using Ampli Taq Gold. Nested PCR for *pol*-RT was performed with one cycle at 94°C for

TABLE 1. CHARACTERISTICS OF HIV-1 STRAINS ISOLATED FROM MOTHERS AND THEIR CHILDREN IN NORTHERN VIETNAM

Sample ID		Drug resistance mutations			Coreceptor usage	Distance within each subject (SE)	
		NRTI	NNRTI	PI		Nucleotide	Amino acid
HN23	M	None	None	None	R5	0.25 (0.14)	1.98 (0.46)
	C-0	n/a	n/a	n/a	n/a		
	C-1	n/a	n/a	n/a	n/a		
	C-3	n/a	n/a	n/a	n/a		
	C-6	n/a	n/a	Fail	R5		
HN46	M	n/a	n/a	n/a	n/a	0.11 (0.09)	0.00 (0.00)
	C-0	None	<i>V106I</i>	None	R5/X4		
	C-12	None	None	None	X4		
HN47	M	None	None	None	R5	1.37 (0.34)	2.57 (0.79)
	C-0	n/a	n/a	n/a	n/a		
	C-1	None	None	None	R5		
HN51	M	n/a	n/a	None	R5	0.07 (0.04)	0.00 (0.00)
	C-0	None	None	None	R5		
	C-1	None	Y181C	None	R5		
HN60	M	None	None	None	R5	0.00 (0.00)	0.00 (0.00)
	C-0	n/a	n/a	n/a	n/a		
	C-1	None	None	None	n/a		
	C-3	None	None	None	R5		
HN88	M	None	None	None	R5	0.52 (0.25)	1.35 (0.60)
	C-0	n/a	n/a	n/a	n/a		
	C-3	None	None	None	R5		
HP13	M	None	None	None	R5	0.83 (0.28)	1.67 (0.74)
	C-0	n/a	n/a	n/a	n/a		
	C-3	None	None	None	R5		
HP25	M	None	None	None	R5/X4	0.07 (0.05)	0.21 (0.16)
	C-0	None	None	None	R5		
	C-3	None	Y181C	None	R5		

M, mother; C, children; C-0, at delivery; C-1, 1 month; C-3, 3 months; C-6, 6 months; C-12, 12 months after birth; NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, nonnucleoside reverse transcriptase inhibitors; PI, protease inhibitor; None, no resistance mutation was detected. *Italics*, minor mutations; **Bold**, major mutation; R5, CCR5; X4, CXCR4; Fail, PCR (+) but population sequencing was not successful; n/a, no PCR amplification. Distance is expressed as the percent nucleotides or amino acid.

1 min and 35 cycles at 98°C for 10 s, 55°C for 30 s (for DRRT7L/DRRT6L primer set) or 45°C for 30 s (for KT101/K102 primer set), and 68°C for 5 min using KOD FX.^{23–25}

The HIV-1 *env* C2V3 region (366 bases) was amplified using the primers JA167mod/JA170mod in the first round and JA168mod/JA169mod in the second round. Amplification was performed with one cycle of 95°C for 2 min and 40 cycles of 95°C for 30 s, 55°C for 30 s, and 68°C for 1 min, with a final extension of 72°C for 10 min.²⁶

The amplified products were detected by staining the gel with ethidium bromide after electrophoresis. The amplified products of the PR and RT regions were sequenced directly with BigDye Terminator v1.1 on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). The amplified products of the *env* C2V3 region were cloned using the TOPO TA kit (Invitrogen) as described previously.²⁷ Eleven to 42 clones were sequenced for each sample using BigDye Terminator v1.1 on a 3500XL sequencer (Applied Biosystems).

Drug resistance

HIV-1 *pol* PR and RT sequences were analyzed for drug resistance mutations using the Stanford University HIVdb sequence analysis program (<http://hivdb.stanford.edu/pages/algs/HIVdb.html>) and the International AIDS Society-USA March 2014 list.^{28,29} Drug resistance variants were defined as

the presence of one or more major resistance-related mutations as specified by the consensus mutation figures of the International AIDS Society-USA.²⁹

Coreceptor usage

HIV coreceptor usage was predicted based on the net charge of amino acids in the *env* V3 region, which was calculated by subtracting the number of acidic amino acids [aspartate (D) and glutamate (E)] from the number of basic amino acids [lysine (K) and arginine (R)]. A net charge less than +5 in the V3 region has been shown to predict CCR5 usage (R5 virus), whereas a net charge of more than +5 has been shown to predict CXCR4 usage (X4 virus), and a net charge equal to +5 indicates R5 variants unless accompanied by an arginine or lysine residue at position 11 or 25 of the V3 amino acid sequences.³⁰

Phylogenetic analysis

A phylogenetic tree was constructed based on the HIV-1 *env* C2V3 region to elucidate the genetic variations between the mothers' and their children's viruses using Clustal W (version 1.83) with the neighbor-joining method, and its reliability was estimated by 1,000 bootstrap replications. The distance of the *env* C2V3 region at each time point in each subject and between mother and infant pairs was analyzed by Mega5.³¹

Results

HIV-1 vertical transmission and drug resistance mutations

Of the eight HIV-1-infected children, four were positive for HIV-1 RNA at birth and the remaining four were negative

at birth and became positive for HIV-1 RNA 1 (HN47 and HN60), 3 (HN88), or 6 (HN23) months after birth (Figs. 1 and 2 and Tables 1 and 2). All HIV-1 strains detected in the mothers and their infants were CRF01_AE (data not shown), although HIV-1 RNA was not amplified in the sample from one mother (HN46). In the HIV-1 strains detected in the

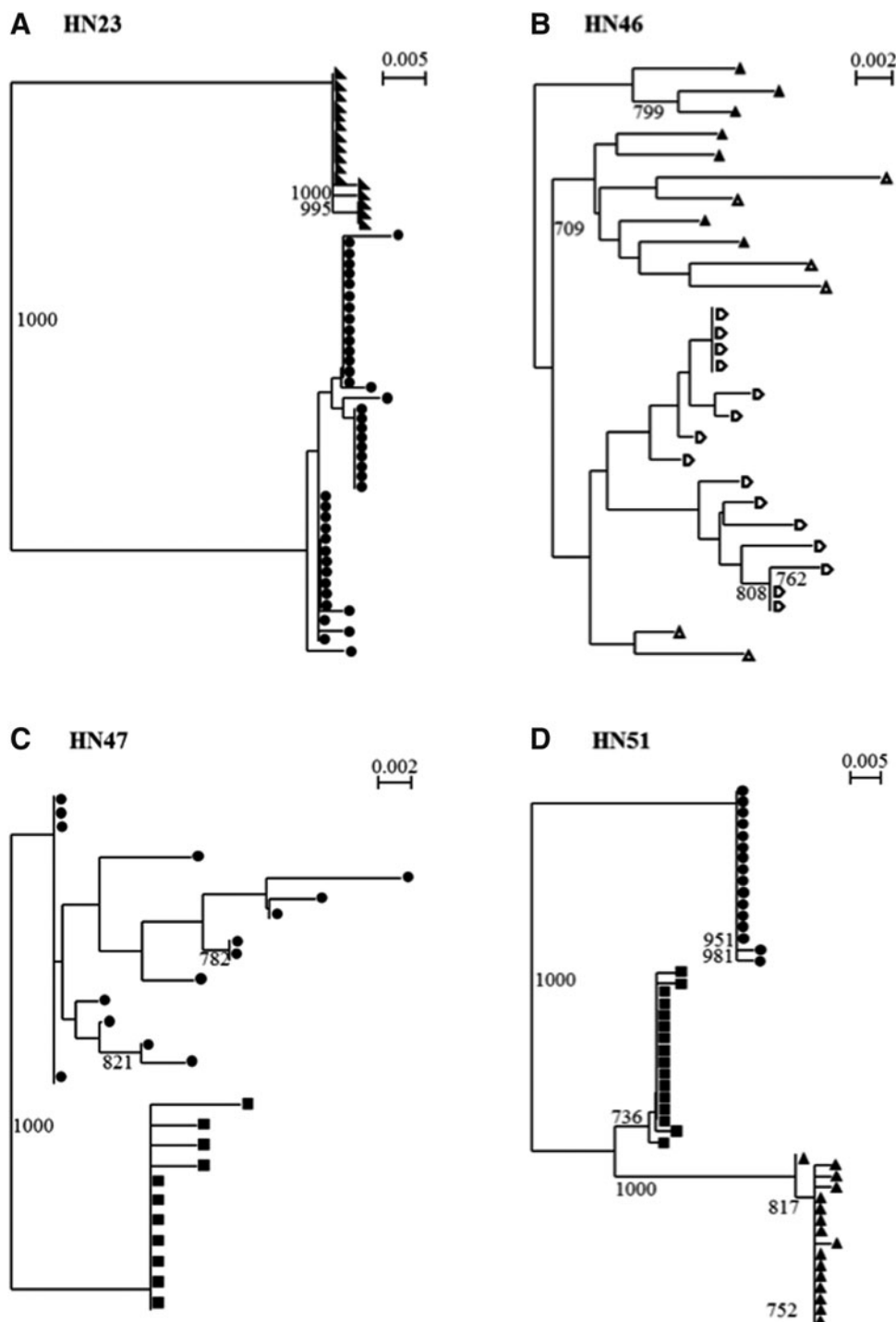


FIG. 2. Phylogenetic trees (A–H) of HIV-1 *env* C2V3 nucleotide sequences in children and/or their mothers. The bootstrap values of 1,000 replicates above 70% are indicated next to the node. ●: mother's virus, R5 variant; ○: mother's virus, X4 variant; ▲: child's virus at birth, R5 variant; △: child's virus at birth, X4 variant; ■: child's virus 1 month after birth, R5 variant; ◆: child's virus 3 months after birth, R5 variant; ▴: child's virus 6 months after birth, R5 variant; ◇: child's virus 12 months after birth, X4 variant.

(Continued →)

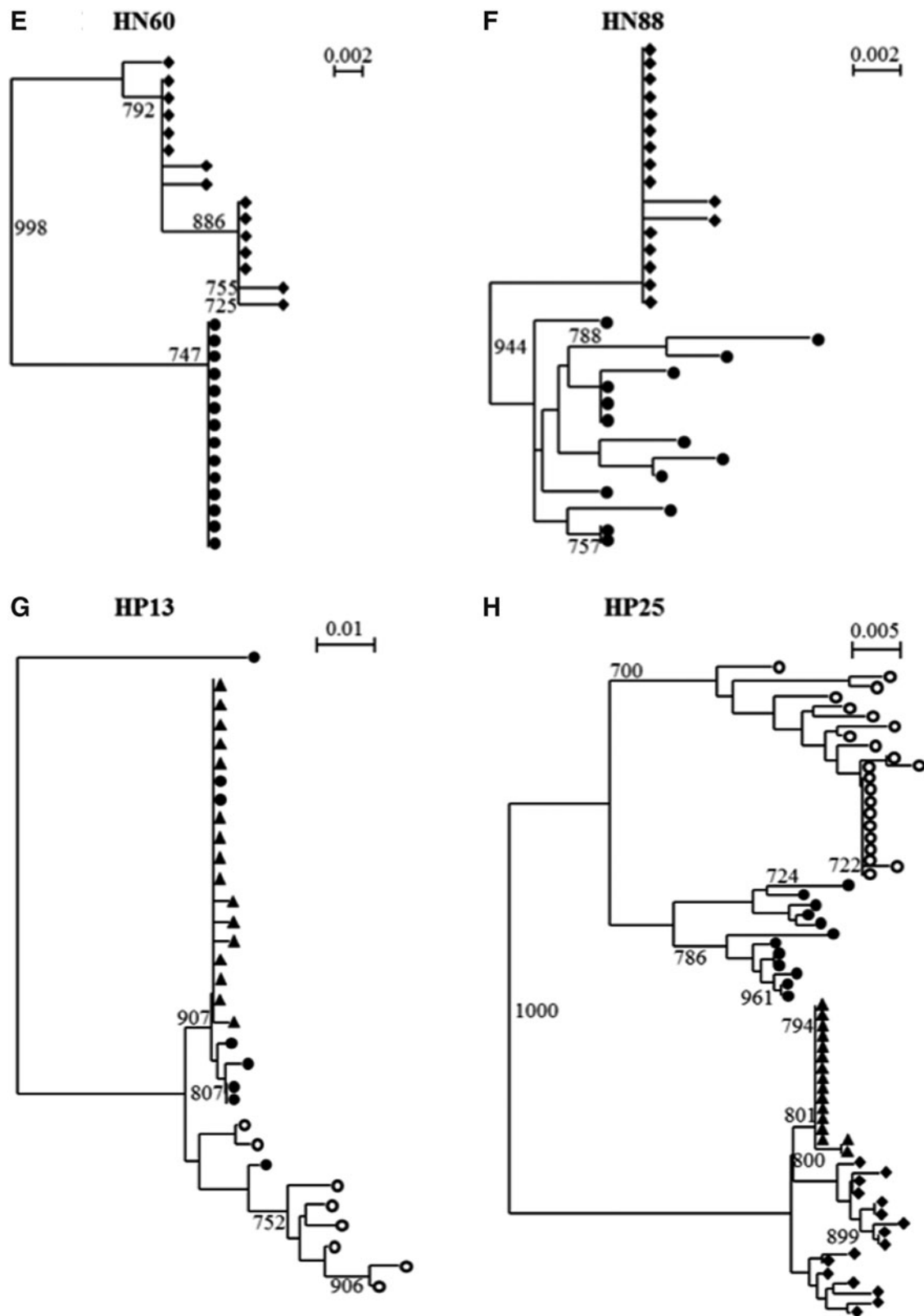


FIG. 2. (Continued)

TABLE 2. V3 AMINO ACID SEQUENCE ALIGNMENT AND PREDICTED CORECEPTOR USAGE OF HIV-1 STRAINS ISOLATED FROM MOTHERS AND THEIR CHILDREN IN NORTHERN VIETNAM

Sample ID	V3 amino acid sequence										Number of clones (total)	Net charge	11/25 amino acid	Predicted phenotype		
	11					25										
Consensus C T R P S N N T R T S I T I G P G Q V - F Y R T G D I I G D I R K A Y C																
HN23																
M	VP	T	31 (42)	3	S/D	R5		
	VP	T	H	9	4	S/D	R5		
	.A	VP	T	H	1	4	S/D	R5		
	VP	T	T	1	2	S/D	R5		
C-6	N	16 (16)	3	S/D	R5		
HN46																
C-0YD	K	K	V	RK	-Y	E	T	2 (13)	5	K/E	X4*	
Y	K	A	RK	-Y	E	T	2	6	K/E	X4	
YE	K	K	V	RK	-Y	E	T	1	5	K/E	X4*	
YD	G	K	V	RK	-Y	E	R	1	5	K/E	X4*	
YE	G	K	A	RK	-Y	E	T	1	4	K/E	R5	
Y	K	Q	V	RK	-Y	E	T	2	5	Q/E	R5	
YE	E	K	V	RK	-Y	E	T	1	3	K/E	R5	
YE	K	K	A	RK	-Y	T	E	T	1	4	K/E	R5	
YE	Q	K	A	RK	-Y	E	T	1	4	K/E	R5	
Y	K	Q	V	RK	-Y	T	E	T	1	4	Q/E	R5	
	C-12Y	N	K	A	RK	-Y	E	T	5 (15)	5	K/E	X4*
	Y	E	K	A	RK	-Y	K	T	1	6	K/K	X4
.....YK		E	K	A	RK	-Y	K	T	3	7	K/K	X4	
.....Y		N	K	A	RK	-Y	K	T	3	7	K/K	X4	
.....YK		E	K	A	RK	-Y	E	T	2	5	K/E	X4*	
.....YKAE		K	A	RK	-Y	K	T	1	7	K/K	X4		
HN47																
M	M	G	7 (15)	4	S/G	R5		
	.I	M	G	7	4	S/G	R5		
C-1	M	I	1	3	S/D	R5		
	M	G	8 (11)	4	S/G	R5		
	MN	G	1	4	S/G	R5		
	.F	M	G	1	4	S/G	R5		
.....	M	D	G	1	3	S/G	R5		
HN51																
M	VA	16 (16)	3	S/D	R5		
C-0	L	16 (16)	3	S/D	R5		
C-1	VA	16 (16)	3	S/D	R5		
HN60																
MK	T	L	K	14 (14)	4	S/D	R5		
C-3K	T	L	N	V	F	7 (15)	5	S/N	R5		
K	T	L	F	7	4	S/D	R5		
K	T	L	A	F	1	4	S/D	R5		
HN88																
MK	G	E	13 (14)	4	G/E	R5		
K	G	V	E	1	4	G/E	R5		
C-3K	G	E	16 (16)	4	G/E	R5		
HP13																
MI	R	M	K	E	6 (16)	5	R/E	X4*		
Y	I	R	M	K	E	2	5	R/E	X4*		
	G	E	6	3	G/E	R5		
I	G	E	1	3	G/E	R5		
I	L	K	1	3	S/D	R5		
C-0	G	E	16 (16)	3	G/E	R5		
HP25																
M	K	V	M	R	E	R	20 (34)	6	K/E	X4		
	K	V	M	R	E	1	5	K/E	X4*		
	.T	K	V	M	R	E	R	1	5	K/E	X4*	
	N	R	E	10	4	S/E	R5		
	N	R	E	R	2	5	S/E	R5		
	M	M	16 (16)	3	S/D	R5		
C-0	G	M	6 (16)	3	G/D	R5		
S	M	5	3	S/D	R5		
	G	M	K	3	3	G/D	R5		
S	M	K	2	3	S/D	R5		
	
	

A net charge of less than and more than +5 in the V3 region indicated CCR5-using (R5) and CXCR4-using (X4) variants, respectively, and a net charge of +5 indicated R5 variants * unless accompanied by an arginine (R) or lysine (K) residue at position 11 or 25 of the V3 amino acid sequence.

mothers, no reverse transcriptase inhibitor (RTI) or protease inhibitor (PI) resistance mutations were found. No ARV resistance mutations were detected in the children at birth, but Y181C, a nonnucleoside reverse transcriptase inhibitor (NNRTI) resistance mutation, appeared in two (25%) children 1 (HN51) and 3 (HP25) months after birth, respectively. No NRTI or PI resistance mutations were found in these children during the follow-up period (Table 1).

Genetic diversity of HIV-1 in the mother–children pairs

Clonal sequencing of the HIV-1 *env* C2V3 region was performed and the genetic distance of the clones within each subject was calculated (Table 1). The mean genetic distance of the nucleotide sequences in the mothers ranged from 0.00% to 2.82% (median value: 1.09%) and from 0.06% to 2.05% (median value: 0.50%) in the children. In five pairs, the children's sequences had less diversity than their mother's sequences. In two pairs, the children's sequences had higher diversity than their mother's sequences (0.17% at birth and 0.14% 1 month after birth vs. 0.07% in HN51 and 0.52% vs. 0.00% in HN60). Thus, the children's sequences were generally more homogeneous than their mothers' sequences.

In the child HP25, the viral genetic diversity increased from 0.06% at birth to 0.89% 3 months after birth, whereas in the child HN51 it decreased slightly from 0.17% at birth to 0.14% 1 month after birth.

Predicted HIV-1 coreceptor usage

The multiple alignments of the amino acid sequences of the HIV-1 *env* V3 region and the predicted coreceptor usage of HIV-1 strains isolated from the eight mother-and-child pairs are shown in Table 2. Five mothers and their children (HN23, HN47, HN51, HN60, and HN88) harbored R5 viruses. Two mothers (HP13 and HP25) harbored both R5 and X4 viruses, whereas their children harbored only R5 viruses. In the remaining one mother (HN46), HIV-1 RNA could not be amplified and her child harbored both R5 and X4 viruses at birth, but only X4 virus 12 months after delivery.

*Phylogenetic analysis of HIV-1 *env* C2V3 regions*

To examine the viral genetic relationship between each mother and her child, we performed phylogenetic analyses based on the HIV-1 *env* C2V3 nucleotide sequences (Fig. 2). In five mother–child pairs (HN23, HN47, HN88, HP13, and HP25), the HIV-1 strains of the children formed more homogeneous clusters than the strains of the mothers (Fig. 2A, C, and F–H), whereas in the HN51 and HN60 pairs the mothers' viruses formed more homogeneous clusters than the children's viruses (Fig. 2D and E). In the HP25 child, the viral population was very homogeneous at birth, but its diversity spread significantly 3 months after birth (Fig. 2H). In the HN51 pair, the child's viruses formed two independent clusters, at birth and 1 month after birth. Interestingly, the child's virus population 1 month after birth was much closer to the mother's virus population than the child's virus population at birth (Fig. 2D). In the HP13 and HP25 pairs, the mothers' virus populations consisted of both R5 and X4 viruses, whereas their children's consisted of only R5 viruses, even though X4 viruses were dominant in their mothers (Fig. 2G and H). In the remaining pair, HN46, in which maternal HIV-1 RNA was not amplified, the child's viruses exhibited high di-

versity at birth as well as 12 months after birth, and the virus population at birth consisted of both R5 and X4 viruses (Fig. 2B).

Discussion

In the current study, we investigated the appearance and profile of ARV resistance mutations, the predicted coreceptor usage, and the genetic diversity of the HIV-1 strains isolated from eight pairs of HIV-1-infected mothers and their children in Northern Vietnam. All of the children were given single-dose nevirapine at birth and followed for up to 12 months. This is the first molecular epidemiological study of vertical HIV-1 infection in Vietnam.

Of the eight HIV-1-infected children, four were positive for HIV-1 RNA at birth, suggesting *in utero* transmission of HIV-1, and four children became positive 1, 3, and 6 months after birth, suggesting intrapartum transmission of HIV-1. All of the children were fed formula. Therefore, most of the children who failed PMTCT of HIV-1, even with single-dose nevirapine within 48 h after birth, might be infected with HIV-1 *in utero* and intrapartum.

The HIV-1 strains harboring a major nevirapine resistance mutation, Y181C, appeared in two (25%) of the eight children 1 and 3 months after birth. This finding is consistent with a previous study in Ethiopia that reported a prevalence of 26% for the drug resistance mutation in children who received single-dose nevirapine from 2001 to 2007, and that Y181C was the most dominant mutation in the children.³² Other studies have reported nevirapine resistance mutations among 33–38% of HIV-infected children when both the children and their mothers were exposed to single-dose nevirapine.^{33–36}

In the current study, the nevirapine resistance mutation was not detected in the children at birth, and no ARV resistance mutations were detected in their mothers, suggesting that the drug resistance mutation developed in the children independently after nevirapine administration. These findings completely agree with previous reports that most resistance mutations to nevirapine in children are selected *de novo* when the virus is exposed to nevirapine rather than being transmitted from their mothers.¹⁶ Our findings together with previous results strongly suggest that longer treatment with ARV, such as 4–6 weeks of once-daily nevirapine or twice-daily zidovudine, which is recommended in the WHO ART guidelines 2013,³⁷ should be considered as a PMTCT of HIV-1 in the children, instead of single-dose nevirapine. It is also suggested that ARV resistance testing is necessary for the children who failed PMTCT of HIV-1 before starting antiretroviral therapy. To the best of our knowledge, this is the first report of the appearance and profile of ARV-resistant HIV-1 in children given single-dose nevirapine at birth for PMTCT in Vietnam.

In the present study, two children (HP13 and HP25) born to mothers who had both R5 and X4 viruses harbored only R5 viruses at birth, despite the X4 viruses being dominant in the mothers, demonstrating selected transmission of R5 virus from mother to child. These findings are consistent with previous studies in which the R5 phenotype was predominantly transmitted from mother to child.^{38,39} In contrast, one child (HN46) harbored both R5 and X4 viruses at birth with relatively high diversity, suggesting that multiple HIV-1 strain transmission occurred *in utero* and evolved to some extent by delivery. However, whether HIV infection was initiated with both R5 and X4 viruses or only R5 virus(es) that evolved *in utero* to

produce X4 virus populations in this child cannot be determined because no data were available on the mother's virus and X4 and R5/X4 viruses can be transmitted from mother to child before, during, or shortly after delivery.⁴⁰

In the current study, we found that the viral populations of the children were more homogeneous than those of their mothers at delivery, suggesting "bottleneck transmission" of HIV-1 variants from mother to child.³¹ However, in the two mother-child pairs, the children's viral populations at birth and 1 month (HN51) and 3 months (HN60) after birth exhibited higher genetic diversity than their mothers' viral populations, suggesting recent infection of the mothers with HIV-1 and rapid viral replication and evolution in these children after birth. This is in agreement with a study from Sweden in which the children's viral populations were more heterogeneous than the mothers' viral populations.³¹

Phylogenetic analyses showed that single-strain infection occurred in five of the eight mother-child pairs, few-strain infection in two, and multiple-strain infection in one during vertical transmission. This finding is consistent with previous studies on MTCT of HIV-1 showing that either single or multiple variants can be transmitted vertically,⁴²⁻⁴⁴ but multiple-strain infection during vertical transmission seems to be less frequent than infection initiated by a single or a few closely related maternal variants.⁴¹⁻⁴⁷ Phylogenetic analyses also showed relatively longer genetic distances between the viral populations of mothers and their children in all pairs, except for HP13, which could be due to the transmission of a minor variant from the mother to her child.⁴⁸

In the current study, evolution of HIV-1 strains (HP25), viral population shifts (HN46 and HN51), and switch of coreceptor usage from R5 to X4 (HN46) were also observed in the children. However, the small number of HIV-1-infected mother-child pairs and incomplete sample collection from the children during the follow-up are acknowledged limitations in this study. Multicentric cohort studies are necessary to confirm these findings and to investigate the mechanism and risk factors associated with HIV-1 vertical transmission in Vietnam.

Sequence Data

The sequences obtained in this study were deposited in GenBank under accession numbers KP006809 to KP007125 for *env* and KP007126 to KP007143 for *pol*.

Acknowledgments

This study was supported by the Ministry of Education in Vietnam (the 322 scholarship program). We are grateful to all participants enrolled in this study at Hanoi and Haiphong Hospitals. We thank our colleagues: Dr. Ha, Ms. Ngoc (National Institute of Hygiene and Epidemiology, Hanoi, Vietnam), Ms. Thuy (National Hospital of Pediatrics, Hanoi, Vietnam), and the members of the Viral Infection and International Health Department of the Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan.

Author Disclosure Statement

No competing financial interests exist.

References

- UNAIDS/WHO: Global report: UNAIDS report on the global AIDS epidemic 2012. UNAIDS 2012. www.unaids.org.
- The Working Group on Mother-To-Child Transmission of HIV: Rates of mother-to-child transmission of HIV-1 in Africa, America, and Europe: Results from 13 perinatal studies. *J Acquir Immune Defic Syndr Hum Retrovirol* 1995;8(5):506-510.
- European Collaborative Study: Risk factors for mother-to-child transmission of HIV-1. *Lancet* 1992;339(8800):1007-1012.
- Coovadian HM, Rollins NC, Bland RM, *et al.*: Mother-to-child transmission of HIV-1 infection during exclusive breastfeeding in the first 6 months of life: An intervention cohort study. *Lancet* 2007;369(9567):1107-1116.
- John GC and Kreiss J: Mother-to-child transmission of human immunodeficiency virus type 1. *Epidemiol Rev* 1996;18(2):149-157.
- Kourtis AP, Lee FK, Abram EJ, *et al.*: Mother to child transmission of HIV-1: Timing and implications for prevention. *Lancet Infect Dis* 2006;6(11):726-732.
- Deng H, Liu R, Ellmeier W, *et al.*: Identification of a major co-receptor for primary isolates of HIV-1. *Nature* 1996;381(6584):661-666.
- Berger EA, Doms RW, Fenyo EM, *et al.*: A new classification for HIV-1. *Nature* 1998;391(6664):240.
- Casper CH, Clevestig P, Carlenor E, *et al.*: Link between the X4 phenotype in human immunodeficiency virus type 1-infected mothers and their children, despite the early presence of R5 in the child. *J Infect Dis* 2002;186(7):914-921.
- Nyamache AK, Muigai AW, Ng'ang'a Z, and Khamadi SA: Profile of HIV type 1 coreceptor tropism among Kenyan patients from 2009 to 2010. *AIDS Res Hum Retroviruses* 2013;29(8):1105-1109.
- Salvatori F and Scarlatti G: HIV type 1 chemokine receptor usage in mother-to-child transmission. *AIDS Res Hum Retroviruses* 2001;17(10):925-935.
- Scarlatti G, Hodara V, Rossi P, *et al.*: Transmission of human immunodeficiency virus type 1 (HIV-1) from mother to child correlates with viral phenotype. *Virology* 1993;197(2):624-629.
- Mofenson LM and Munderi P: Safety of antiretroviral prophylaxis of perinatal transmission for HIV-infected pregnant women and their infants. *J Acquir Immune Defic Syndr* 2002;30(2):200-215.
- Newell ML and Thorne C: Antiretroviral therapy and mother-to-child transmission of HIV-1. *Expert Rev Anti Infect Ther* 2004;2(5):717-732.
- Jackson JB, Musoke P, Fleming T, *et al.*: Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: 18-month follow-up of the HIVNET 012 randomised trial. *Lancet* 2003;362(9387):859-868.
- Eshleman SH, Mracna M, Guay LA, *et al.*: Selection and fading of resistance mutations in women and infants receiving nevirapine to prevent HIV-1 vertical transmission (HIVNET 012). *AIDS* 2001;15(15):1951-1957.
- UNAIDS/WHO: AIDS epidemic update. UNAIDS 2007. http://data.unaids.org/pub/EPISlides/2007/2007_epiupdate_en.pdf. 2007.
- Nguyen TA, Oosterhoff P, Ngoc YP, *et al.*: Barriers to access prevention of mother-to-child transmission for HIV positive women in a well-resourced setting in Vietnam. *AIDS Res Ther* 2008;5(7):1-12.
- Ministry of Health, Viet Nam administration of HIV/AIDS control: Viet Nam HIV/AIDS estimates and projections 2007-2012. Ha Noi 2009.

20. Ministry of Health, Socialist Republic of Viet Nam: Guidelines for Diagnosis and Treatment of HIV/AIDS. 2005 (No. 06/2005/QĐ-BYT).
21. Viet Nam National Committee for AIDS, Drugs and Prostitution Prevention and Control: Viet Nam AIDS Response Progress Report 2012. Following Up the 2011 Political Declaration on HIV/AIDS. Ha Noi 2012. www.unaids.org/en/dataanalysis/knowyourresponse/countryprogressreports/2012countries/ce_VN_Narrative_Report.pdf.
22. Ha TT, Anh NM, Bao NH, *et al.*: HIV-1 mother-to-child transmission, post-test counselling, and antiretroviral prophylaxis in Northern Viet Nam: A prospective observational study. *Scand J Infect Dis* 2012;44(11):866–873.
23. Lihana RW, Lwembe RM, Bi X, *et al.*: Efficient monitoring of HIV-1 vertically-infected children in Kenya on first-line antiretroviral therapy. *J Clin Virol* 2011;52(2):123–128.
24. Ishizaki A, Cuong NH, Thuc PV, *et al.*: Profile of HIV type 1 infection and genotypic resistance mutations anti-retroviral drugs in treatment-naïve HIV type 1-infected individuals in Hai Phong, Viet Nam. *AIDS Res Hum Retroviruses* 2009;25(2):175–182.
25. Phan TTC, Ishizaki A, Phung DC, *et al.*: Characterization of HIV type 1 genotypes and drug resistance mutations among drug-naïve HIV type 1-infected patients in Northern Vietnam. *AIDS Res Hum Retroviruses* 2010;26(2):233–235.
26. Mild M, Björndal A, Medstrand P, and Fenyö EM: Isolation of human immunodeficiency virus-type 1 (HIV-1) clones with biological and molecular properties of the primary isolate. *Virology* 2006;350(1):58–66.
27. Ndembi N, Yumo H, Takehisa J, *et al.*: HIV type 1 infection in Pygmy hunter gatherers is from contact with Bantu rather than from nonhuman primates. *AIDS Res Hum Retroviruses* 2003;19(5):435–439.
28. HIV Drug Resistance Database, Stanford University. February 2014. <http://hivdb.stanford.edu/pages/algs/HIVdb.html>.
29. Wensing AM, Calvez V, Gunthard HF, *et al.*: 2014 Update of the drug resistance mutations in HIV-1. *Top Antivir Med* 2014;22(3):642–650.
30. Lwembe R, Lihana RW, Ochieng W, *et al.*: Changes in the HIV type 1 envelope gene from non-subtype B HIV type 1-infected children in Kenya. *AIDS Res Hum Retroviruses* 2009;25(2):141–147.
31. Nowak P, Karlsson AC, Naver L, *et al.*: The selection and evolution of viral quasiespecies in HIV-1 infected children. *HIV Med* 2002;3(1):1–11.
32. Persaud D, Bedri A, Zierniak C, *et al.*: Slower clearance of nevirapine resistant virus in infants failing extended nevirapine prophylaxis for prevention of mother-to-child HIV transmission. *AIDS Res Hum Retroviruses* 2011;27(8):823–829.
33. Arrivé E, Newell ML, Ekouevi DK, *et al.*: Prevalence of resistance to nevirapine in mothers and children after single-dose exposure to prevent vertical transmission of HIV-1: A meta-analysis. *Int J Epidemiol* 2007;36(5):1009–1012.
34. Stringer JS, McConnell MS, Kiarie J, *et al.*: Effectiveness of non-nucleoside reverse-transcriptase inhibitor-based antiretroviral therapy in women previously exposed to a single intrapartum dose of nevirapine: A multi-country, prospective cohort study. *PLoS Med* 2010;7(2):1–13.
35. Moorthy A, Gupta A, Bhosala R, *et al.*: Nevirapine resistance and breast-milk HIV transmission: Effects of single and extended-dose nevirapine prophylaxis in subtype C HIV-infected infants. *PLoS One* 2009;4(1):1–8.
36. Wind-Rotolo M, Durand C, Cranmer L, *et al.*: Identification of nevirapine-resistance HIV-1 in the latent reservoir after single-dose nevirapine to prevent mother-to-child transmission of HIV-1. *J Infect Dis* 2009;199(9):1301–1309.
37. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. June 2013. http://apps.who.int/iris/bitstream/10665/85321/1/9789241505727_eng.pdf.
38. Cavarelli M, Karisson I, Zanchetta M, *et al.*: HIV-1 with multiple CCR5/CXCR4 chimeric receptor use is predictive of immunological failure in infected children. *PLoS One* 2008;3(9):e3292.
39. Salvatori F and Scarlatti G: HIV type 1 chemokine receptor usage in mother-to-child transmission. *AIDS Res Hum Retroviruses* 2001;17(10):925–935.
40. Huang W, Eshleman SH, Toma J, *et al.*: Vertical transmission of X4 tropic and dual tropic HIV-1 in five Ugandan mother-infant pairs. *AIDS* 2009;23(14):1903–1908.
41. Wade CM, Lobidel D, and Brown AJ: Analysis of human immunodeficiency virus type 1 *env* and *gag* sequence variants derived from a mother and two vertically infected children provides evidence for the transmission of multiple sequence variants. *J Gen Virol* 1998;79(5):1055–1068.
42. Wolinsky SM, Wike CM, Korber BT, *et al.*: Selective transmission of human immunodeficiency virus type-1 variants from mothers to infants. *Science* 1992;255(5048):1134–1137.
43. Dickover RE, Garratty EM, Plaeger S, and Bryson YJ: Perinatal transmission of major, minor, and multiple maternal human immunodeficiency virus type 1 variants in utero and intrapartum. *J Virol* 2001;75(5):2194–2203.
44. Scarlatti G, Leitner T, Halapi E, *et al.*: Comparison of variable region 3 sequences of human immunodeficiency virus type 1 from infected children with the RNA and DNA sequences of the virus populations of their mothers. *Proc Natl Acad Sci USA* 1993;90(5):1721–1725.
45. Verhofstede C, Demecheleer E, De Cabooter N, *et al.*: Diversity of the human immunodeficiency virus type 1 (HIV-1) *env* sequence after vertical transmission in mother-child pairs infected with HIV-1 subtype A. *J Virol* 2003;77(5):3050–3057.
46. Ahmad N, Baroudy BM, Baker RC, and Chappey C: Genetic analysis of human immunodeficiency virus type 1 envelope V3 region isolates from mother and infants after perinatal transmission. *J Virol* 1995;69(2):1001–1012.
47. Persaud D, Pierson T, Ruff C, *et al.*: A stable latent reservoir for HIV-1 in resting CD4 (+) T lymphocytes in infected children. *J Clin Invest* 2000;105(7):955–1003.
48. Ceballos A, Andreani G, Ripamonti C, *et al.*: Lack of viral selection in human immunodeficiency virus type 1 mother-to-child transmission with primary infection during late pregnancy and/or breastfeeding. *J Gen Virol* 2008;89(11):2773–2782.

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